GROWTH OF EXCISED RHODODENDRON EMBRYOS IN DIFFERENT TYPES OF MEDIA USED FOR EMBRYO CULTURE

by

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Introductory Statement

This thesis has been written in manuscript form to be submitted for publication in the Journal of the American Society for Horticultural Science. The research was done in fall 1978 and in spring 1979 using immature and mature rhododendron and azalea embryos. The experiments were conducted in the laboratories of the Department of Horticulture, Kansas State University.

Growth of Excised Rhododendron Embryos in Different Types of Media Used for Embryo Culture[1]

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Abstract. Immature and mature embryos of all 4 rhododendron and azalea gardening class types were excised and cultured in the dark at 25°C on 5 types of media used for embryo culture (Norstog, Berlyn, Raghaven, Murashige, and Emsweller). Embryo size was measured at 1, 2, 3, 4, 6, 8 and 12 weeks to see the change across time. Small embryos of 0.5-1 mm in length from all rhododendron and azalea classes did not grow or germinate on the 5 media. Embryos of mature deciduous azalea species and hybrids larger than 1 mm in length, particularly R. calendulaceum, showed the most embryo growth and differentiation leading to some germination on Raghaven and Emsweller media. Embryos matured by cold treated immature seeds at 3-4°C until pods became brown and split-open, grew much better than mature embryos in culture after excision. Mature embryos of 3 month cold treated immature R. calendulaceum seeds grew best on Raghaven and Emsweller even though just a low percent germination occurred on Raghaven. Norstog and Emsweller appeared to support embryo growth of 3 month cold treated immature R. atlanticum seeds without any germination. Mature embryos of 25 days cold treated R. mollis x mollis seeds grew

and developed successfully on Raghaven, Emsweller, and Murashige with high percent germination; they had slight growth on Norstog and Berlyn but a few embryos germinated late on Berlyn. The mature embryos of species and hybrids of deciduous azalea differ in their nutritional requirements due to their own genetic characteristics, but generally they seemed to respond best on simple media as Emsweller. Rhododendron tissue seemed to be sensitive to the concentrations of salts, compounds in the medium especially Murashige, which caused browning to small rhododendron seedlings after one month exposure to light intensity of 700-1,000 ft.c.

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Embryo culture is a term used to describe the growth of embryos in various culture media regardless of age, size and developmental stage at the time when embryos are excised out of their natural environment. The technique of growing excised plant embryos in vitro, using salt solution as well as organic nutrients, has been studied for many years. During recent years, embryo culture has been applied for two main objectives, to get viable hybrids from crosses difficult or impossible from seeds, and to overcome dormancy in seeds (11). Failure of hybrid seed may be due to poor endosperm development or incompatibility of endosperm with embryo; the embryo may have been viable if removed early enough (15).

Maheshwari and Swamy, Narrayanaswami and Norstog, and Rappaport (11, 15, 23) reviewed the use of embryo culture to obtain viable hybrids of interspecific crosses of different plants or to shorten the breeding cycle of the plants that require several years for germination from seeds. Shahin, et al. (25) obtained interspecific hybrids of Zinnia peruviana and Z. elegans through embryo culture. Recently Zilis and Martin (30) reported that Paeonia and Viburnum seedlings can be produced in a relatively short time through embryo culture.

Much work on embryo culture has been done to aid interspecific hybridization of lily species. Emsweller, Asen and Uhring (4) excised and grew embryos of Lilium speciosum x L. auratum on artificial medium in order to prevent the toxins of the endosperm from killing the hybrid embryos and to provide nutrition unavailable from the defective endosperm. They reported that it might be possible to obtain hybrids of lily

plants by excising embryos from seeds at stages prior to full maturity and growing them on nutrient agar in small test tubes. Their results indicated the probability that some substance in the seed attacked and destroyed the embryo. In other work (4) they isolated from the seeds three acids, ferulic, P-coumaric, and sinapic, of which ferulic acid is known to be a growth inhibitor. North and Willis (19) and North (20) obtained successful cultures of embryos of interspecific crosses of Lilium lankogense. Stimart and Ascher (26) cultured embryos from intraspecific crosses of Lilium longiflorum at different stages of growth on 5 different media used for embryo culture. They noticed Norstog medium stimulated growth of immature and nearly mature excised embryos followed by 40% germination; Emsweller medium increased the growth of mature embryos followed by 4.1% germination; on the other media of Berlyn and Miksche, Raghaven and Torrey, and Murashige and Skoog, they grew slowly with no germination. They concluded that for continued growth leading to germination of L. longiflorum embryos, a transfer is necessary from Norstog medium to the simple medium of Emsweller; but this transfer depends on the cross, since small embryos from L. x 'Damson' x L. longiflorum germinated without a transfer from Norstog medium.

Results of research using immature embryos of different plants vary from complete failure of further embryo development to germination and growth into a normal seedling. Generally the success or failure of the immature embryo development depended on the species of the plant, the culture medium used and the age

at the time the embryo was excised out of the seed. In fact the embryo culture cannot be considered successful until young embryos reach a stage of tissue mass and proper differentiation and are able to produce normal germination. Some workers failed to get immature embryos to grow using the basal medium of White (10, 12). Others succeeded in growing very young embryos only when the basal medium was supplemented with growth promoting substances (12, 13, 21, 22, 28, 29). Young embryos are more susceptible to osmotic shock than mature embryos, so the nutritive medium suitable for older embryos is often not beneficial for younger stages (13, 17, 24).

There are no reports in the literature on rhododendron embryo culture. Rhododendron belongs to the heath family (Ericaceae) which is widely represented in eastern Asia and eastern and western North America, as well as other parts of the world with temperate climate. The Rhododendron genus includes two distinct gardening groups, the azaleas and rhododendrons. Botanists have divided the genus into many sections and subgenera and have placed azaleas into sections separate and apart from the great numbers of true rhododendron (6).

Rhododendrons are classified into two main groups, scaly-leaved and non-scaly-leaved. The azaleas are classified into two classes, deciduous and evergreen, and they are all non-scaly, having small hairy leaves. Fruitful breeding is limited to species and hybrids within the classes of rhododendrons and azaleas. Hybrids between deciduous and evergreen azaleas are difficult, but not impossible (7). It is unlikely that crosses between scaly and non-scaly rhododendron are productive. Almost

no seeds are produced or if seeds are produced they are not generally viable. Leach (9) explained that it could be from the incompatibility of pollen and stigma surface or the pollen after germinating could not extend its tube far enough down a long style to affect fertilization. Kho and Baer (8) studied pollen tube growth and fertilization in the cross R. impeditum (scaly) x R. williamsianum (smooth). They found that pollen germinated on the stigma and pollen tubes grew normally in the style, but difficulties occurred when the pollen tubes reached the ovary; the majority of the tubes could not penetrate into the embryo sacs of the ovules. In some cases pollen tube growth is not a problem but seeds do not develop because of endosperm abortion or because of embryo abortion and lethal inheritance from the different plants. Embryo culture might be a useful means to get hybrids from difficult and impossible crosses between Rhododendron classes that give no seed.

The present study investigated the response of excised immature and mature embryos of various species and hybrids from all Rhododendron classes on the 5 different types of media used for embryo culture by Stimart and Ascher (26).

Materials and Methods

Immature capsules of rhododendron and azalea species and hybrids (open-pollinated) were collected early in October 1978 from Meade Park Gardens, Topeka, Kansas, and University of Minnesota Landscape Arboretum, Chaska, Minnesota. Those capsules were green and not split. During 18-24 October 1978 the small immature embryos were cultured from the 4 gardening types of rhododendron: evergreen azalea, scaly rhododendron, non-scaly rhododendron and deciduous azalea (listed in Table 2).

Mature seeds of rhododendron species and hybrids were received on 12 January 1979 from the American Rhododendron Society, Mercer Island, Washington. The mature embryos were excised and cultured on 18-26 January 1979 from the 4 classes of rhododendron (listed in Table 2).

Immature capsules of deciduous azalea species and hybrids collected in Minnesota in October 1978 matured in the cold temp of a refrigerator (3-4^{o}C), became brown and split-open. R. mollis x mollis capsules were matured after 25 days cold treatment; mature embryos from seeds in these capsules were excised and cultured on 28 October 1978. R. calendulaceum and R. altanticum embryos were excised and cultured on 28-30 January 1979 after 3 months in the refrigerator (listed in Table 2).

Media preparation:

Stock solutions of the chemical contents of each medium were made first to be used for medium mixing. The chemicals

were weighed accurately using an electric analytic balance (Mettler H18, No. 504341) dissolved with the help of magnetic stirring or, in some cases, with heat. They were added to the solution in the order listed in the tables, with the next one added after the previous element was clearly dissolved (Appendix). The stock solutions were stored in the refrigerator (3-4^{o}C) to be used again after 3 months for the mature embryo experiment.

Culture medium on which rhododendron embryos were placed are representative of five types of media used for embryo culture (Table 1, and Appendix).

1. Norstog medium (Norstog, 1973):
   A filter sterilized complete medium containing vitamins, amino acids, as well as major and trace elements.
2. Berlyn medium (Berlyn and Miksche, 1965, from Nitsch, 1951):
   A simple autoclaved medium containing major and trace elements.
3. Raghaven medium (Raghaven and Torrey, 1963):
   A relatively simple autoclaved medium containing major and trace elements supplemented with vitamins.
4. Murashige medium (Murashige and Skoog, 1962):
   A much used autoclaved medium containing major and trace elements supplemented with vitamins.
5. Emsweller medium (Emsweller, Asen and Uhring, 1962):
   A simple autoclaved medium containing macro elements.

For all media the added ingredients were sucrose and agar. The added amount of agar was 6 g/l in all media based on the

work of Stoltz (27) suggesting that embryo growth is restricted by higher agar concentrations. The solution of Norstog medium containing amino acids was first filter sterilized through a 0.22 um type GS millipore membrane in a sterile laminar flow hood, and then added to the autoclaved component of agar solution. The medium was poured into autoclaved 8 dram glass shell vials in the sterile laminar flow hood, filling the vials one-third full. The vials were covered with aluminum foil caps. The other media were poured into clean vials filling them one-third full, capped with aluminum caps, and autoclaved for 20 min at 15 lb pressure and 121°C. The caps were made of 3"x3" square pieces of aluminum foil sheets. After pouring and autoclaving, the vials containing the liquid medium were slanted so as to form a slanted surface on the medium. The vials sat at room temp for a week and were examined for contamination before being used for culturing. There was no contamination in the vials except one vial of Norstog medium.

Embryo Culturing:

Immature capsules of all rhododendron species and hybrids were sterilized by submerging for 30 min in a 1:10 dilution of household bleach (sodium hypochlorite) in water. Then, they were submerged briefly in 95% ethanol and placed in a sterile laminar flow hood. The hood space and all unautoclaved materials placed into the hood including hands and arms were swabbed with 95% ethanol. Face masks were also worn. Sterile capsules were cut open inside the hood and the immature seeds removed from the capsules and placed in a petri plate containing sterilized

deionized distilled water. The embryos were excised from the seeds using a dissecting microscope and two single-edged, spear-shaped needles. The needles were dipped in 95% ethanol before each use. Two embryos were placed on the agar slant per vial. After the embryos were transferred, the vials were capped and sealed with parafilm; they were enclosed in polyethlene to reduce drying and incubated at 25°C in the dark.

The sterilization procedure was not effective enough for some species and hybrids of rhododendron since 50% were contaminated after a week of culture. The following technique of sterilization was used. The capsules were submerged in 10% household bleach for at least one hr. After submerging for a while in 95% ethanol, they were opened inside the sterile laminar flow hood. The seeds were removed from the capsules and placed for 10 min in 10% household bleach, in a sterilized petri plate. Then, the seeds and the bleach were poured onto a sterilized filter paper in a sterilized funnel and washed several times with sterile deionized distilled water. The filter paper containing the seeds was taken out of the funnel and laid in a sterilized petri plate, thus keeping the seeds wet. By this method, it was easier to excise the embryos and the contamination was reduced to less than 2%.

Mature seeds were sterilized by the second technique, the seeds being placed directly into 10% household bleach for 10 min.

There were a total of 324 immature and 660 mature excised embryos. For each of 6 immature and 11 mature species and

hybrids of each rhododendron class for each of the 5 media, there were 6 vials containing a total of 12 embryos. Immature embryos of the non-scaly rhododendron class were cultured only on two media (Norstog and Emsweller).

Since the medium seemed to be drying out faster than expected even though a tray of water was placed in the incubator, it was necessary to wet the surface of the medium by injecting sterilized deionized distilled water into the vials through the aluminum foil caps with a disposable syringe sterilized by gamma radiation.

When embryos produced a root and at least one true leaf, they were considered germinated and were removed from the dark incubator to be placed on a shelf under a fluorescent light of 700-1,000 ft. c. (16 hr. days).

Embryo size was measured for each class or species cultured at the time of excision using an occular micrometer and the power 3x on a binocular microscope. Measurements of embryo size were taken weekly for the first month them every other week in the second month and finally after three months of culturing.

RESULTS

Size of embryos varied at culturing; immature embryo size ranged for the various species from an average of 0.51 mm to 1.03 mm in length and 0.14 mm to 0.21 mm in width where the mature ones ranged from 0.60 mm to 1.08 mm in length and 0.15 mm to 0.25 mm in width. Those that matured in refrigerator ranged from 0.96 mm to 1.60 mm in length and 0.21 mm to 0.24 mm in width (Table 2).

Small immature embryos of 0.5-1 mm of all species and hybrids of all rhododendron classes did not grow well on the 5 tested media (Table 3 and 4). Embryos of mature evergreen azalea, scaly rhododendron and non-scaly rhododendron also had no growth on the 5 media from the first to the twelfth week (Table 5); some deciduous azalea embryos did grow but there were differences in embryo growth response among the species and hybrids on all 5 media across time (Table 6). R. calendulaceum embryos increased more than 1 mm in length on Raghaven and Emsweller but did not show significant growth on the other media; the embryos responded best in growth on Raghaven (Fig 1, 2, and 3). R. atlanticum embryos did not increase in size on any media. Embryos of R. 'Night Light' showed some development on Emsweller medium and slight development on Norstog and Raghaven, but no growth on Berlyn or Murashige.

Embryos of R. calendulaceum and R. atlanticum (same seed source as for the immature species) cultured after 3 months cold treatment for the seeds in refrigerator (3-4^{o}C) had good

growth on Emsweller, Raghaven and Norstog and some development on Berlyn but slight growth on Murashige (Table 7). The most embryo growth of R. calendulaceum was on Raghaven but R. atlanticum embryos showed best development on Norstog or Emsweller. Embryos from the hybrid R. mollis x mollis, cultured from mature seeds after 25 days of cold, grew best on Murashige, Raghaven, and Emsweller, but had slight non-significant growth on Berlyn and Norstog (Fig 4, 5, and 6). Embryo size was measured only to the 6th week on Raghaven, Murashige, and Emsweller, since more than 40% of embryos germinated. The last embryo measurements were taken the 8th week on Norstog and Berlyn since there was no further embryo growth and development.

Table 8 contains the percent germination for those deciduous azalea species that responded best, the tested media, the time (week) when the embryo germinated and the embryo dimensions at the time of germination. Some embryos of R. mollis x mollis seeds matured in the cold germinated on all tested media except Norstog, with the highest percent germinated (41.7) on Emsweller or Raghaven and the lowest (16.7) on Berlyn. Some of these embryos growing on Emsweller germinated as early as three weeks after culture, with four to six weeks needed for those that germinated on Raghaven and Murashige, and 8 weeks for the few embryos that germinated on Berlyn. Embryo size was longest on Murashige (5.82 mm) and widest on Emsweller (0.58 mm) at the time of germination. Embryo length was noticeably shorter on Berlyn (2.96 mm) and also the narrowest on Raghaven (0.37 mm). One embryo of mature R. calendulaceum

germinated after 3 weeks of culture on Emsweller and another after 4 weeks on Raghaven. The embryo was larger on Raghaven than Emsweller at the time of germination. One embryo from R. calendulaceum seeds matured in the cold germinated on Raghaven after 3 weeks of culture.

Embryo growth of all immature and mature deciduous azalea species and hybrids were compared to determine the difference among all 5 tested media from the 1st to the 12th week for natural immature and mature embryos (Table 9, 10), and from the 1st to the 6th week for matured embryos in cold treated immature seeds (Table 11). Embryos of immature species and hybrids of deciduous azalea did not grow in length from the 2nd to the 12th week on any of the 5 tested media (Table 9). Embryo width showed slight significant difference among the tested media. On the 8th and 12th week, there was a significant difference in width between embryos on Raghaven and Murashige media. Mature embryo length of all deciduous azalea species and hybrids was significantly different among all 5 tested media from the 1st week to the 12th week (Table 10). In the first week embryo length was significantly shorter on Norstog medium. On the 8th and 12th week, embryo length was best on Raghaven, less well on Emsweller and Berlyn, and least well on Murashige and Norstog. Embryo width was not different among all 5 media from the 1st to the 12th week.

Embryo length and width of all the species and hybrids of deciduous azalea matured by the cold in the refrigerator did not differ among the tested media at the first week, but

did differ by the 6th week (Table 11). It appeared that embryo growth was best on Emsweller and Raghaven, less good on Murashige and Norstog and least good on Berlyn.

Discussion

Stimart and Ascher (26) found immature lily embryos (.14-.6 mm in length) cultured on the same five media increased slightly on all media but grew most on Norstog. However, Lofland (10) failed to grow immature cotton embryos excised at 15 days of age (4 mm) on the basal medium of White, and those excised at 20 days (7-8 mm) grew very slowly, and produced many abnormal forms. He found that only mature embryos excised at 27 days of age (10 mm) grew rapidly on the basal medium. Also he found growth-stimulating substances ineffective in promoting rapid growth of cotton embryos. Matsubara (12) found very young embryos (.15 mm length) of Datura tatula grew very poorly in basal medium lacking any growth promoting substance. Van Overbeek (28) was able to obtain seedlings of Datura stramonium from young embryos as small as 0.15 mm in length only when coconut milk was added to the medium referring to the growth promoting substance found in coconut milk as "embryo factor." Norstog (16) stated that a medium containing 90% coconut milk stimulated the growth of even the smallest embryos cultured, but he noticed inhibition of root development in the medium which contained autoclaved coconut milk. Similarly Berlyn and Miksche (2) observed that autoclaved coconut milk had no statistical effect on the growth of pine embryos. Many researchers observed the value of the addition of casein hydrolysate as a nutrient for embryo growth (10, 13, 29). Raghaven and Torrey (21, 22) indicated the important role of some growth substance

in development of embryos through various stages in their ontogeny. They showed that supplementing the basal medium with indoleacetic acid, kinetin, and adenine sulfate permitted successful development of globular embryos of Capsella (< 80 micron long) in vitro, and the high concentration of sucrose or salt in the medium partially replaced the effect of the growth factors in inducing development of the globular embryos. The work of Rietsema (24) showed that the smallest embryos of Datura stramonium need higher concentrations of sucrose; those approximately 0.2-0.35 mm long need at least 2%, those 0.15 mm in length need 8% and those 0.1 mm in length need 12%. The study of Mauney (13) indicated that the most important single modification of the basic medium for successful culture of cotton embryos was the adjustment of the osmotic pressure. Young cotton embryos (0.1-0.2 mm) could be cultured successfully on White's medium, which has a normal osmotic value of approximately 2 atm, if this value was increased to approximately 10 atm by the addition of 8% sucrose to the 2% sucrose in the medium. He found also that the addition of 0.7% NaCl maintained growth longer and embryos over a wider range of sizes could be cultured. Also the embryos needed to be transferred to a medium with intermediate osmotic level (0.3% NaCl) after they reached 2-3 mm (3-4 weeks) and, after the development of embryos on this medium for 1-2 weeks, they had to be transferred to a medium containing no NaCl to permit germination of the embryo at a low osmotic pressure. Norstog (17) showed that the survival of very small embryos of Hordeum vulgare (200-400 micron) could be achieved by increases in the concentrations of potassium salts in the basic White medium. He stated that

the reduction of sucrose concentrations to approximately 6.8% both eliminated the initial "osmotic shock" to embryos and minimized the "precocious germination" in most cultures maintained in constant light of 500-100 ft.c.

In general, small embryos, having a highly concentrated sap in their embryonic tissue, are shocked and contracted when cultured on a medium containing low concentrations (13). Those same small embryos cultured on a medium containing higher concentrations than within their sap are also shocked due to the increase of concentration in the sap which would cause toxicity and death of embryonic tissues.

The failure of those mature embryos of the classes of evergreen azalea, scaly rhododendron, and non-scaly rhododendron on all 5 tested media may be explained due to the small size of these embryos (0.6 mm in length). Although the embryos of R. sutchuenense, a non-scaly rhododendron, are rather large, they showed slow growth and reacted like the other small mature embryos. In contrast, only mature embryos larger than 1 mm in length from deciduous azaleas showed best response in embryonic growth and development of all the Rhododendron species used, especially on Raghaven and Emsweller. The simple ingredients and concentration in Emsweller and Raghaven may satisfy the needs of those mature embryos. The addition of vitamins in Raghaven seemed to stimulate more growth than Emsweller. Raghaven and Torrey (21) indicated that older embryos of Capsella need simple nutritional requirements; the development of embryos is sustained by amino acids and growth factors available

within the embryos themselves, or the embryos can synthesize them from the mineral salts and sucrose provided in the medium. They showed that growth and differentiation in the cultured embryos are regulated not by the osmotic condition of the culture medium, but by specific constituents of the medium which may act by their control of differential cell division and cell enlargement during development. Also they suggested that mature embryos produce the necessary stimulus for the growth of the primary root system characteristic of the adult plant when grown either in light or dark conditions, but younger embryos need to be cultured in dark since, if cultured in light, the stimulus is lost or not formed in adequate amount. This agrees with Norstog (18) who noticed that light tends to inhibit germination of cultured barley embryos.

Emsweller, et al. (4) grew embryos into plants from mature capsules of Lilium speciosum x L. auratum and observed that the cotyledon emerged first and a root was not formed until 2-4 mm of cotyledon had developed. The advance in the growth of some mature rhododendron embryos on Emsweller medium agreed with the results of Stimart and Ascher (26) who noticed that some mature lily embryos germinated on Emsweller after one month of culture. The low percentage germination on Raghaven and Emsweller may be due to damaged growing points initials of some embryos during excision or to the lack of growth factors in the tissue of some of those embryos or probably to the change in pH of the medium which could not support differentiation of the embryos. The non-germinated embryos increased in size

showing abnormal growth with brown tissue mass; a few formed callus.

Cold storage seems to be a good means for accelerating growth of embryos in immature seeds. Embryos in immature seeds of species and hybrids of deciduous azalea matured in the cold grew successfully in culture after they were excised. It might be that this cold treatment helped the embryos to break their dormancy faster and grow to a larger size because of a faster rate of growth after excision and culturing. Vernalization, possibly another name for this cold treatment, is known to consist of a shortening of life cycle by hastening each cell in turn through its various stages of development (3). Gregory and Purvis (5) vernalized embryos of winter rye excised from the grain and succeeded in accelerating germination on agar containing glucose and mineral nutrients. They concluded that the process of vernalization is localized in the embryo itself and germination is not dependent on vernalization of the endosperm or aleurone layer, but on the response of the embryo to cold treatment which is able to synthesize hormones from a simple medium containing glucose and inorganic salts.

Norstog (17) observed that filter-sterilization of certain organic components (amino acids) including glutamine, together with the addition of ammonium malate, produced increased growth in size of cultured embryos. Stimart and Ascher (26) found that Norstog medium supported rapid growth of immature and mature hybrid lily embryos and gave high percentage of germination.

Mature embryos in cold treated R. mollis x mollis seeds have larger sizes and so many of them germinated on Raghaven, Emsweller, Murashige and a few on Berlyn but none on Norstog.

The complex Norstog medium seemed not satisfactory to the needs of those hybrid embryos. The simple media of Emsweller and Raghaven with 2% sugar concentration supported more growth and germination than Murashige which has more salt and higher sugar concentrations. Berlyn has a rather simple medium with 4% more sugar than the other media.

Several media will need to be designed to meet the different requirements of the different species and hybrids of deciduous azalea. Embryos of various species need different nutritional requirements due to their genetic characteristics.

Most of the media seemed to have high concentrations of some chemicals toxic to late stages of embryonic development and seedlings. After a month of exposing germinated embryos of the hybrid R. mollis x mollis to light intensities of approximately 1,000 ft. c. (16 hrs photoperiod) for continuous growth, most seedlings lost their green color and turned brown followed by weakness or death. Especially affected were embryos cultured on Murashige medium. The browning could be due to the high light intensity since the change to a brown color occurred on seedlings of R. calendulaceum exposed to lower light intensities of approximately 700 ft.c. The browning might be due to the high salt concentration in the medium. Anderson (1) found that rhododendron tissue in contact with Murashige rapidly turned brown and that browning moved up the seedling stem until the

cultures were completely killed. He eliminated the browning problem by reducing the nitrogen containing compound KNO_3 to one-half since the potassium concentration was toxic to rhododendron tissue.

For future research needs, the media need to be modified to contain ingredients and concentration which satisfy the needs of small embryos from all rhododendron and azalea classes that failed to grow on the basal media. The media may need to be supplemented with a growth promoting substance such as coconut milk or casein hydrolysate or indoleacetic acid, kinetin or adenine sulfate, to give embryo differentiation especially for those small embryos which cannot produce enough growth factors within their tissue or have a tendency to diffuse into surrounding medium. In larger embryos, enough of these substances accumulate to initiate differentiation so they can be grown in a nutrient medium without addition of growth factors (23). None of the 5 media we used had growth regulators added, although a few alternately could have growth regulators added (2, 14, 21, 22). Sugar or salt concentrations of the medium should agree with the osmotic value in the small embryonic tissue. However, very immature rhododendron embryos (smaller than 0.5 mm in length) need to be tried on both basal and modified media to see if there is possibility of getting successful cultures from those small embryos. As the embryos advance in growth on a high concentration medium, transferring to another medium containing low concentrations seems to be necessary for trying to achieve successful culture (13, 26). Also an optimum pH value needs to be maintained in the medium without shifting throughout the whole experiment by the use of a phosphate buffer. We

should also use different temp in growing rhododendron embryos to see if 25°C is the optimal temp for embryonic growth. Even though most of the embryo cultures grown by various investigators are kept in dark incubators until the necessity of a light source for photosynthetic activity for continuous growth of young seedlings, trials should be done to culture small rhododendron embryos in light. According to the work of Mauney (13), darkness does not seem to be the best environment for excised immature cotton embryos. Embryos cultured in darkness developed chlorophyll only slowly in the cotyledons when transferred to a lighted chamber, but those cultured in approximately 100 ft.c. fluorescent light showed no abnormal effects and developed chlorophyll immediately after being excised and cultured. Those unsuccessful cultures of immature rhododendron embryos cultured in the dark became white or turned brownish.

Other work needs to be tried on vernalizing rhododendron embryos after excision to know if they will respond in growth better than those given cold treatment in the seeds and then excised and cultured. For rhododendron embryo culture, it would be necessary to look for the media whose ingredient and concentrations are not only optimal in achieving best embryonic growth and development but to support later the growth of formed seedling without any toxic effect. However, light intensity less than 700-1,000 ft.c. needs to be tried for small rhododendron seedlings to investigate the optimal needs of light.

Literature Cited

1. Anderson, W. C. 1975. Propagation of Rhododendron by tissue culture; Part 1. Development of a culture medium for multiplication of shoots. Comb. Proc. Intr. Pl. Prop. Soc. 25:129-135.

2. Berlyn, G. P. and J. P. Miksche. 1965. Growth of excised pine embryos and the role of the cotyledons during germination in vitro. Amer. J. Bot. 52:730-736.

3. Cholodny, N. G. 1936. On the Theory of Yarovization. C. R. Acad. U.R.S.S. (N.S.), iii. 391.

4. Emsweller, S. L., S. Asen, J. Uhring. 1962. Lilium speciosum x Lilium auratum. Lily Yb., N. Am. Lily Soc. 15:7-15.

5. Gregory, F. G. and O. N. Purvis. 1938. Studies in vernalisation of cereals. II. The vernalisation of excised mature embryos and of developing ears. Ann. Bot. 2:237-250.

6. Hume, Harold H. Azaleas kinds and culture. 1949. The Macmillan Company, New York, 199p.

7. Kehr, A. E. 1974. Breeding rhododendron and azaleas. In: Clayberg, C. D. et al (eds). Breeding Plants for Home and Garden. Brooklyn Botanic Garden Record, Plants and Gardens. 30(1):55-58.

8. Kho, Y. O. and J. Baer. 1970. A microscopical research on the incompatibility in the cross Rhododendron impeditum x R. williamsianum. Euphytica 19:303-309.

9. Leach, D. G. 1959. Rhododendrons of the world. Charles Scribner's Sons, New York. 544p.

10. Lofland, H. B. 1950. In vitro culture of cotton embryos. Bot. Gaz. 111:307-311.

11. Maheshwari, P. and N. S. Ranga Swamy. 1963. Plant tissue and organ culture from the viewpoint of an embryologist, p. 390-417. In: Maheshwari, P. and N. S. Ranga Swamy, eds. Plant Tissue and Organ Culture - A symposium. Dept. of Botany. Univ. of Delhi, Delhi 6, India.

12. Matsubara, S. 1962. Studies on a growth promoting substance "Embryo Factor", necessary for the culture of young embryos of Datura tatula in vitro. Bot. Mag., Tokyo 75:10-18.

13. Mauney, J. R. 1961. The culture in vitro of immature cotton embryos. Bot. Gaz. 122:205-209.

14. Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-497.

15. Narayanaswami, S. and K. Norstog. 1964. Plant embryo culture. Bot. Rev. 30:586-628.

16. Norstog, K. 1956. The growth of barley embryos on coconut milk media. Bull. Torrey Bot. Club 83:27-29.

17. Norstog, K. 1967. Studies on the survival of very small barley embryos in culture. Bull. Torrey Bot. Club 94:223-229.

18. Norstog, K. 1973. New synthetic medium for the culture of premature barley embryos. In Vitro 8(4):307-308.

19. North, C. and A. B. Wills. 1969. Interspecific hybrids of Lilium lankongense Franchet produced by embryo culture. Euphytica 18:430-434.

20. North, C. 1970. New lily hybrids with the aid of embryo culture. Lily Yb., N. Am. Lily Soc. 33:171-173.

21. Raghaven, V. and J. G. Torrey. 1963. Growth and morphogenesis of globular and older embryos of Capsella in culture. Amer. J. Bot. 50:540-551.

22. Raghaven, V. and J. G. Torrey. 1964. Effects of certain growth substances on the grcwth and morphogenesis of immature embryos of Capsella in culture. Plt. Phys. 39(4):691-699.

23. Rappaport, J. 1954. In vitro cultures of plant embryos and factors controlling other growth. Bot. Rev. 20:201-225.

24. Rietsema, S. Satina, and A. F. Blakeslee. 1953. The effect of sucrose on the growth of Datura stramonium embryos in vitro. Amer. J. Bot. 40:538-545.

25. Shahin, S. S., W. F. Campbell, L. H. Pollard, and A. R. Hamson. 1971. Interspecific hybrids of Zinnia peruviana and Z. elegans through embryo culture. J. Amer. Soc. Hort. Sci. 96(3):365-367.

26. Stimart, D. and P. D. Ascher. 1974. Culture medium suitable for growing small excised lily embryos. Lily Yb., N. Am. Lily Soc. 27:77-84.

27. Stoltz, L. P. 1971. Agar restriction of the growth of escised mature Iris embryos. J. Amer. Soc. Hort. Sci. 96(5):681-684.

28. Van Overbeek, J., M. E. Conklin, and A. F. Blakeslee. 1942. Cultivation in vitro of small Datura embryos. Amer. J. Bot. 29:472-476.

29. Ziebur, N. K., R. A. Brink, L. H. Graf, and M. H. Stahman. 1950. The effect of casein hydrolysate on the growth in vitro of immature Hordeum embryos. Amer. J. Bot. 37:144-148.

30. Zilis, M. R. and M. Martin. 1976. Rapid in vitro germination of immature, dormant embryos. Comb. Proc. Intr. Pl. Prop. Soc. 26:272-275.

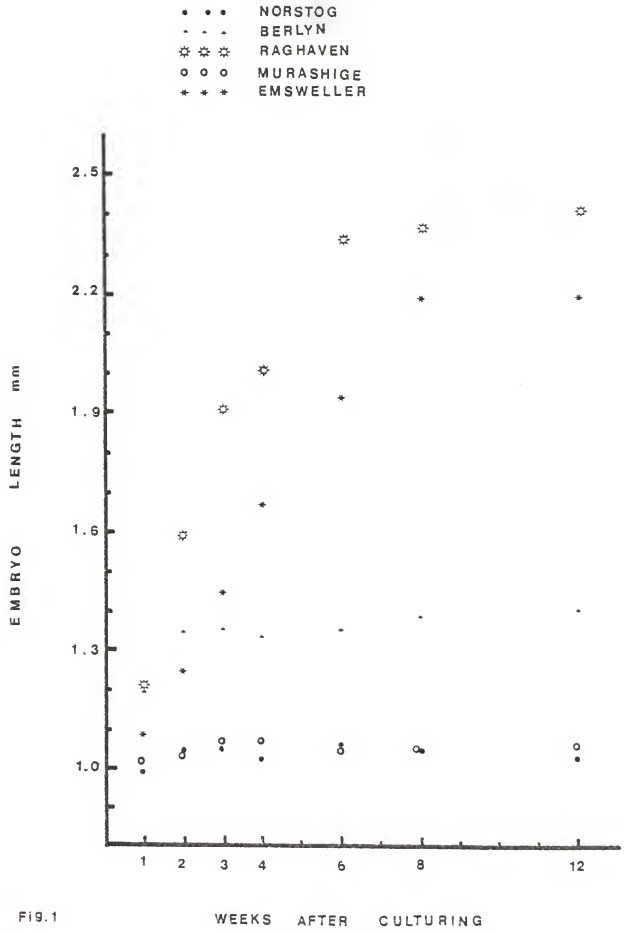

NORSTOG
BERLYN
RAGHAVEN
MURASHIGE
EMSWELLER
EMBRYO LENGTH mm
2.5
2.2
1.9
1.6
1.3
1.0
1
2
3
4
6
8
12
WEEKS AFTER CULTURING

Fig.1

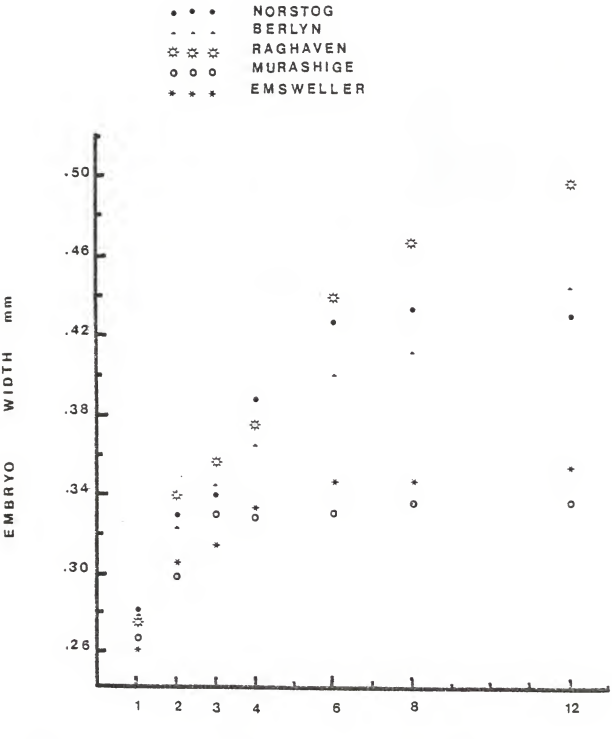

NORSTOG
BERLYN
RAGHAVEN
MURASHIGE
EMSWELLER
EMBRYO WIDTH mm
.50
.46
.42
.38
.34
.30
.26
1
2
3
4
6
8
12
WEEKS AFTER CULTURING

Fig.2

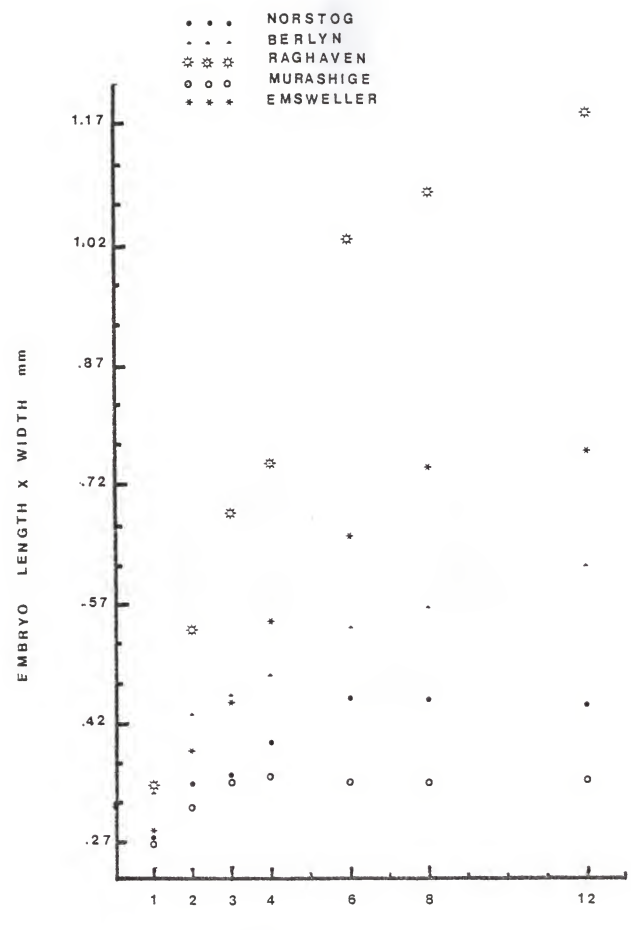

NORSTOG
BERLYN
RAGHAVEN
MURASHIGE
EMSWELLER
1.17
1.02
.87
.72
.57
.42
.27
EMBRYO LENGTH X WIDTH mm
1
2
3
4
6
8
12
WEEKS AFTER CULTURING

Fig.3

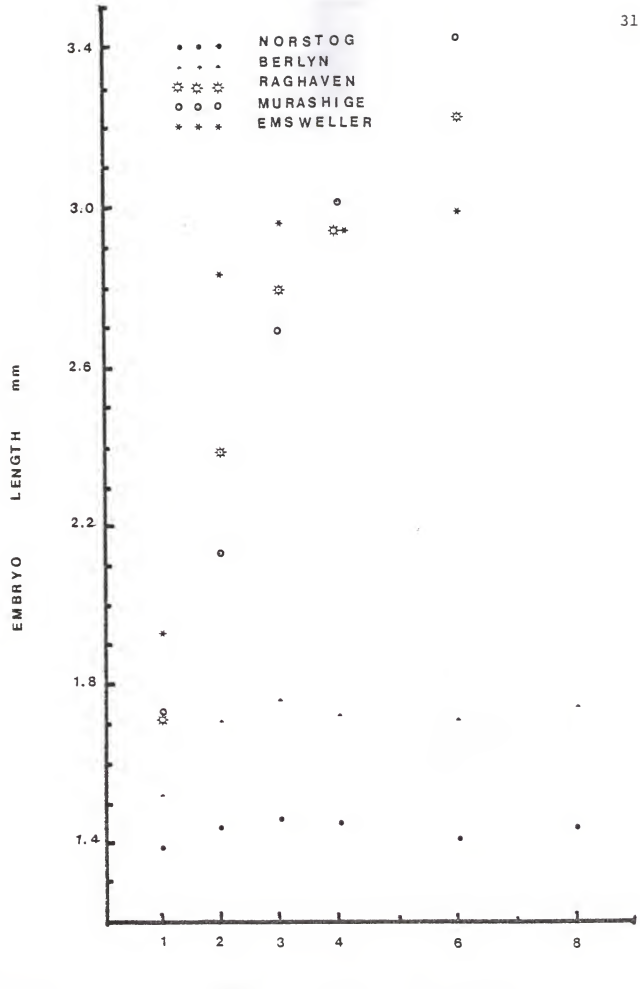

NORSTOG
BERLYN
RAGHAVEN
MURASHIGE
EMSWELLER
EMBRYO LENGTH mm
3.4
3.0
2.6
2.2
1.8
1.4
1
2
3
4
6
8
WEEKS AFTER CULTURING

Fig.4

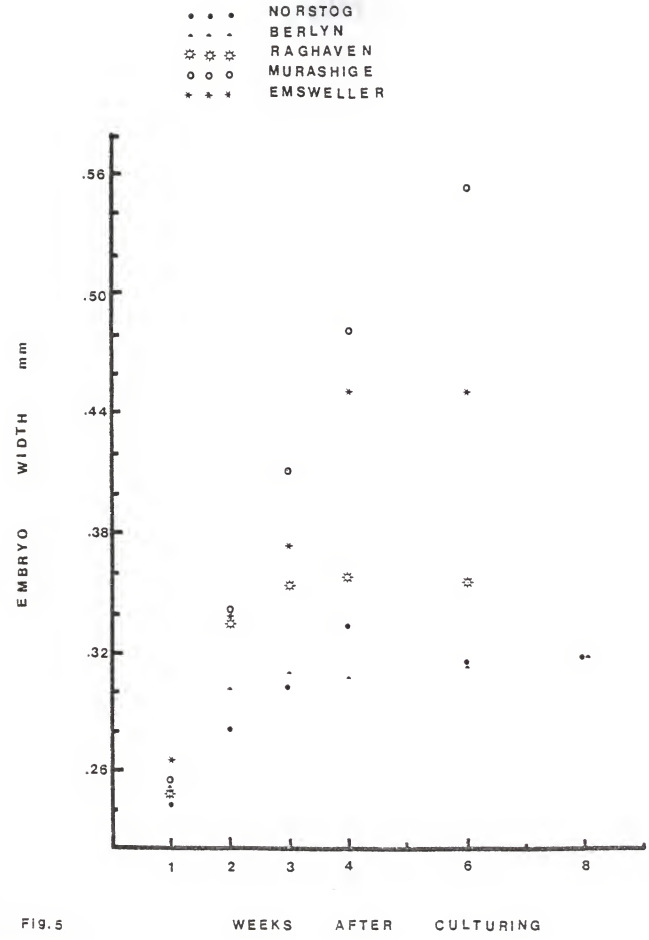

NORSTOG
BERLYN
RAGHAVEN
MURASHIGE
EMSWELLER
EMBRYO WIDTH mm
.56
.50
.44
.38
.32
.26
1
2
3
4
6
8
WEEKS AFTER CULTURING

Fig. 5

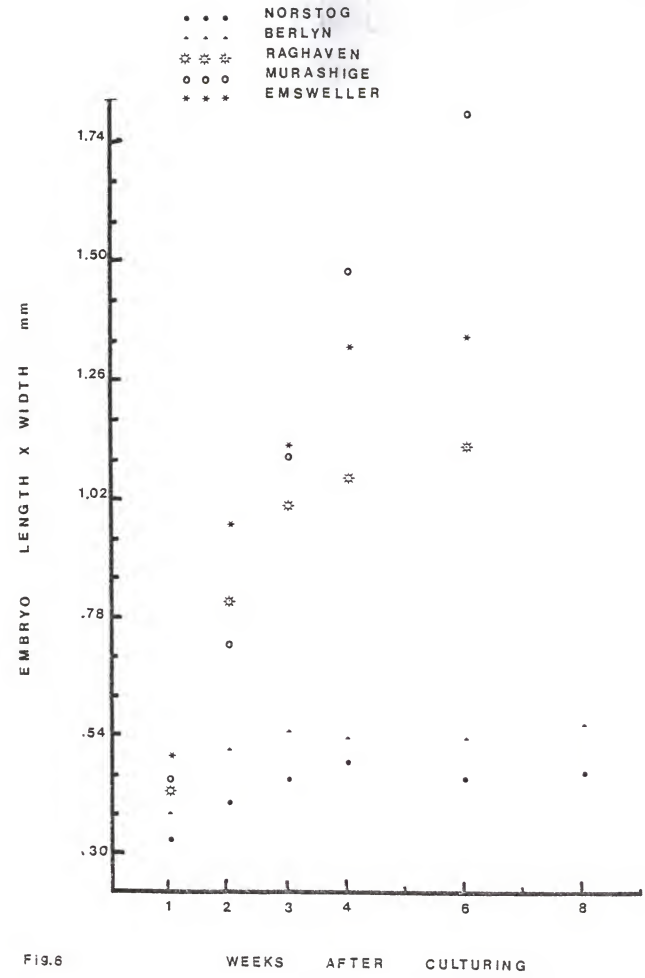

NORSTOG
BERLYN
RAGHAVEN
MURASHIGE
EMSWELLER
EMBRYO LENGTH X WIDTH mm
1.74
1.50
1.26
1.02
.78
.54
.30
1
2
3
4
6
8
WEEKS AFTER CULTURING

Fig.6

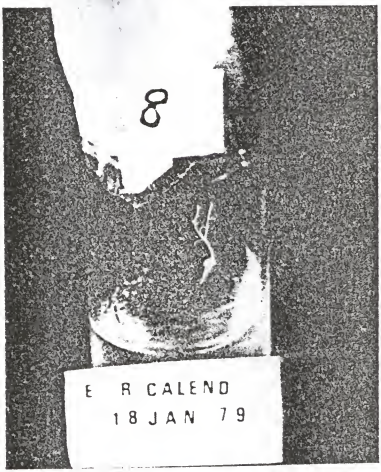


Fig. 7

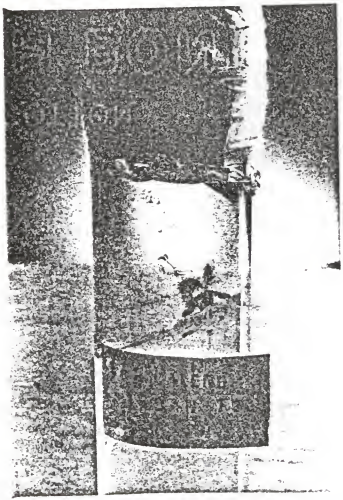
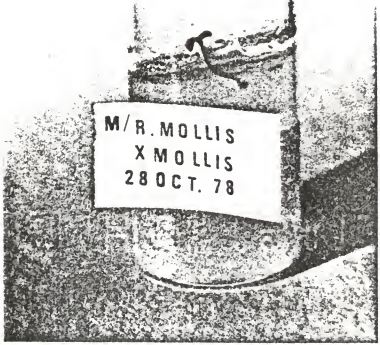


Fig. 8

Fig. 9



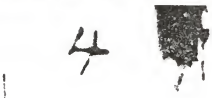


Fig. 10

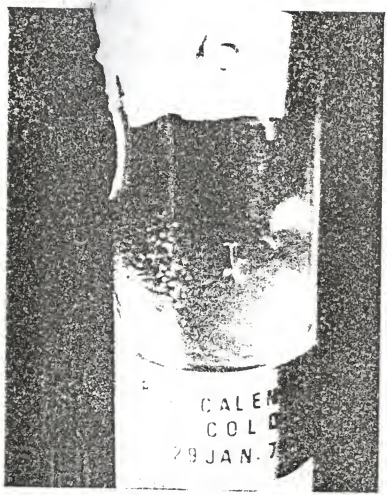


Fig. 11

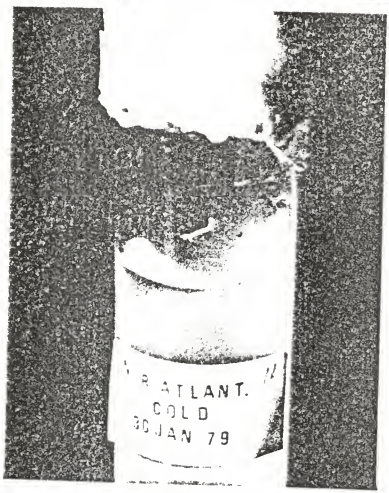


Fig. 12

Table 1. Ingredients in the five media used for culturing excised rhododendron embryos. (Expressed as mg/l unless otherwise noted.)

Components	Medium I (Norstog, 1973)	Medium II (Berlyn & Miksche, 1965)	Medium III (Raghaven & Torrey, 1963)	Medium IV (Murashige & Skoog, 1962)	Medium V (Emsweller, Asen & Uhring 1962)
Major elements:					
KH_2PO_4	910	125	60	170	200
KCl	750		42		
$MgSO_4 \cdot 7H_2O$	740	125	63	370	400
$CaCl_2 \cdot 2H_2O$	740			440	
$Ca(NO_3)_2 \cdot 4H_2O$		500	480		800
KNO_3		125	63	1900	200
NH_4NO_3				1650	
Trace elements:					
$MnSO_4 \cdot 4H_2O$		3.0		22.3	
$MnSO_4 \cdot H_2O$	3.0				
KI				0.83	
H_3BO_3	0.5	0.5	0.56	6.2	
$ZnSO_4 \cdot 7H_2O$	0.5	0.5		8.6	
$CoCl_2 \cdot 6H_2O$	0.025			0.025	
$CuSO_4 \cdot 5H_2O$	0.025	0.025		0.025	
Na_2MoO_4	0.025				
Fe-citrate	10.0		4.52	3.1	
$FeSO_4 \cdot 7H_2O$				27.8	
H_2SO_4, sp.gr. 1.83		0.0005cc/l			
Na_2EDTA				37.3	
$Na_2MoO_4 \cdot 2H_2O$		0.025		0.25	
$MnCl_2 \cdot 4H_2O$			0.36		

Table 1. Ingredients in the five media used for culturing excised rhododendron embryos. (Expressed as mg/l unless otherwise noted.) (Continued)

Components	Medium I (Norstog, 1973)	Medium II (Berlyn & Miksche, 1965)	Medium III (Raghaven & Torrey, 1963)	Medium IV (Murashige & Skoog, 1962)	Medium V (Emsweller, Asen & Uhring, 1962)
Trace elements: (con't)					
$ZnCl_2$			0.42		
$CuCl_2 \cdot 2H_2O$			0.27		
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$			1.55		
Vitamins:					
Pyridoxine-HCl	0.25		0.1	0.5	
Ca-panthothenate	0.25				
Thiamine-HCl	0.25		0.1	0.5	
Inositol (meso)	50.0			100.0	
Nicotinamide				0.5	
Niacin			0.5		
Glycine				2.0	
Amino Acids:					
L-glutamine	400				
L-alanine	50				
L-cysteine	20				
L-arginine	10				
L-leucine	10				
L-phenylalanine	10				
L-tyrosine	10				
Malic Acid	1.0 g/l				
Added ingredients:					
Sucrose	34.2 g/l	40 g/l	20 g/l	30 g/l	20 g/l
pH	4.9	5.5	4.9-5.0	5.7-5.8	
Difco purified Agar	6.0 g/l	6.0 g/l	6.0 g/l	6.0 g/l	6.0 g/l

Table 2. Length and width of immature and mature rhododendron and azalea embryos at the time of excision from seeds.[z]

Rhododendron classes	Length mm	Width mm
Immature:		
Evergreen azalea,[y] Topeka, Kansas	0.56	0.14
Scaly rhododendron:		
R. mucronulatum Turczaninow 58327Y1960,[x] Minnesota	0.60	0.16
Non-scaly rhododendron (smooth), Topeka, Kansas	0.51	0.15
Deciduous azalea:		
R. calendulaceum Torrey 5785B1960, Minnesota	1.03	0.21
R. atlanticum Rehder 5791A1960, Minnesota	0.91	0.17
R. hybrid azalea 56129A1959, Minnesota	0.84	0.17
Mature:		
Evergreen azalea:		
'Delaware Valley White' x 'Glory' #630-1978, hand-pollinated	0.60	0.15
R. scabrum G.Don #154	0.60	0.15
Scaly rhododendron:		
R. augustinii Hemsley #12	0.64	0.16
Non-scaly rhododendron:		
R. maximum x 'sefton' (hybrid) #524, self-pollinated	0.61	0.17
R. sutchuenense Franchet #163	1.08	0.23
Deciduous azalea:		
R. calendulaceum Torrey 1979	1.08	0.26
R. atlanticum Rehder #11	0.68	0.15
R. hybrid azalea 'Night Light'	1.05	0.22
Matured in refrigerator:		
R. calendulaceum Torrey 5785B1960, Minnesota	1.06	0.21
R. atlanticum Rehder 5791A1960, Minnesota	0.96	0.22
R. mollis x mollis 574970C, Minnesota	1.60	0.24

[z] Average length and width of 6 embryos.

[y] All immature and mature species and hybrids from all rhododendron classes are open-pollinated unless otherwise noted.

[x] The numbers followed Minnesota plants are accession numbers at the Arboretum and the numbers followed the mature plant names are the seed numbers listed in the catalog of American Rhododendron Society, Seed Exchange, 1978.

Table 3. Means of the embryo length, width, and length x width (mm) across time of immature embryos of rhododendron classes cultured on all 5 media.[z]

Rhododendron classes	Week	Norstog			Berlyn			Raghaven			Murashige			Emsweller		
		L	W	LW	L	W	LW	L	W	LW	L	W	LW	L	W	LW
Evergreen azalea	1	0.61	0.16	0.0976	0.62	0.16	0.0992	0.49	0.14	0.0686	0.55	0.12	0.0660	0.55	0.14	0.0770
	2	0.68	0.17	0.1156	0.78	0.19	0.1482	0.50	0.14	0.0700	0.56	0.14	0.0784	0.55	0.14	0.0770
	3	0.74	0.19	0.1406	0.84	0.20	0.1680	0.50	0.14	0.0700	0.54	0.13	0.0702	0.54	0.16	0.0864
	4	0.77	0.17	0.1309	0.97	0.22	0.2134	0.52	0.15	0.0780	0.59	0.14	0.0826	0.50	0.16	0.0800
	6	0.87	0.21	0.1827	1.04	0.21	0.2184	0.52	0.16	0.0832	0.59	0.13	0.0767	0.57	0.19	0.1083
	8	0.86	0.21	0.1806	1.07	0.26	0.2782	0.51	0.15	0.0765	0.58	0.13	0.0754	0.59	0.22	0.1298
	12	0.87	0.22	0.1914	0.94	0.26	0.2444	0.53	0.15	0.0795	0.57	0.14	0.0798	0.61	0.22	0.1342
Scaly rhododendron R. mucronulatum	1	0.55	0.17	0.0935	0.69	0.18	0.1242	0.60	0.15	0.0900	0.64	0.16	0.1024	0.65	0.16	0.1040
	2	0.58	0.17	0.0986	0.70	0.20	0.1400	0.70	0.19	0.1330	0.70	0.21	0.1470	0.66	0.16	0.1056
	3	0.60	0.18	0.1080	0.72	0.20	0.1440	0.69	0.21	0.1449	0.72	0.23	0.1656	0.70	0.18	0.1260
	4	0.60	0.17	0.1020	0.72	0.20	0.1440	0.72	0.21	0.1512	0.72	0.23	0.1656	0.66	0.18	0.1188
	6	0.60	0.18	0.1080	0.75	0.25	0.1875	0.74	0.22	0.1628	0.73	0.24	0.1752	0.69	0.19	0.1311
	8	0.64	0.19	0.1216	0.77	0.24	0.1848	0.71	0.24	0.1704	0.73	0.25	0.1825	0.67	0.18	0.1206
	12	0.60	0.18	0.1080	0.75	0.26	0.1950	0.75	0.25	0.1875	0.71	0.24	0.1704	0.72	0.21	0.1512
Non-scaly rhododendron	1	0.52	0.16	0.0832	--	--	--	--	--	--	--	--	--	0.48	0.14	0.0672
	2	0.51	0.16	0.0816	--	--	--	--	--	--	--	--	--	0.49	0.14	0.0686
	3	0.53	0.17	0.0901	--	--	--	--	--	--	--	--	--	0.49	0.15	0.0735
	4	0.53	0.18	0.0954	--	--	--	--	--	--	--	--	--	0.51	0.15	0.0765
	6	0.55	0.18	0.0990	--	--	--	--	--	--	--	--	--	0.48	0.15	0.0720
	8	0.54	0.18	0.0972	--	--	--	--	--	--	--	--	--	0.49	0.15	0.0735
	12	0.52	0.17	0.0884	--	--	--	--	--	--	--	--	--	0.49	0.14	0.0686

[z] Means of 12 embryos of each rhododendron class.

Table 4. Means of the embryo length, width, and length x width (mm) across time of immature azalea species and hybrids cultured on all 5 media.[z]

Deciduous azalea	Week	Norstog			Berlyn			Raghaven			Murashige			Emsweller		
		L	W	LW	L	W	LW	L	W	LW	L	W	LW	L	W	LW
R. calendulaceum	1	0.94	0.22	0.2068	1.09	0.22	0.2398	1.00	0.22	0.2200	0.94	0.16	0.1504	1.12	0.17	0.1904
	2	0.92	0.21	0.1932	1.16	0.23	0.2668	1.22	0.26	0.3172	0.98	0.17	0.1666	1.12	0.18	0.2016
	3	0.93	0.20	0.1860	1.18	0.23	0.2714	1.28	0.27	0.3456	1.00	0.17	0.1700	1.10	0.19	0.2090
	4	0.94	0.21	0.1974	1.13	0.23	0.2599	1.26	0.27	0.3402	0.98	0.17	0.1666	1.10	0.19	0.2090
	6	0.95	0.20	0.1900	1.18	0.24	0.2832	1.29	0.30	0.3870	0.99	0.17	0.1683	1.13	0.20	0.2260
	8	0.90	0.20	0.1800	1.15	0.23	0.2645	1.26	0.30	0.3780	0.99	0.17	0.1683	1.13	0.18	0.2034
	12	0.91	0.20	0.1820	1.16	0.22	0.2552	1.28	0.29	0.3712	0.96	0.16	0.1536	1.12	0.18	0.2016
R. atlanticum	1	0.91	0.22	0.2002	0.94	0.23	0.2162	0.87	0.21	0.1827	0.88	0.15	0.1320	0.93	0.19	0.1767
	2	0.94	0.21	0.1974	0.93	0.22	0.2046	0.85	0.19	0.1615	0.89	0.18	0.1602	0.95	0.19	0.1805
	3	0.92	0.22	0.2024	0.88	0.21	0.1848	0.84	0.20	0.1680	0.89	0.18	0.1602	0.93	0.20	0.1860
	4	0.92	0.23	0.2116	0.91	0.20	0.1820	0.84	0.19	0.1596	0.88	0.18	0.1584	0.96	0.20	0.1920
	6	0.94	0.22	0.2068	0.91	0.21	0.1911	0.90	0.19	0.1710	0.91	0.18	0.1638	0.96	0.19	0.1824
	8	0.90	0.22	0.1980	0.90	0.21	0.1890	0.87	0.20	0.1740	0.89	0.17	0.1513	0.93	0.19	0.1767
	12	0.90	0.21	0.1890	0.90	0.22	0.1980	0.87	0.20	0.1740	0.88	0.19	0.1672	0.88	0.19	0.1672
R. hybrid azalea	1	0.80	0.17	0.1360	0.84	0.21	0.1764	0.69	0.13	0.0897	0.83	0.18	0.1494	0.93	0.18	0.1674
	2	0.83	0.16	0.1328	0.82	0.18	0.1476	0.76	0.15	0.1140	0.90	0.19	0.1710	0.95	0.19	0.1805
	3	0.81	0.16	0.1296	0.81	0.18	0.1458	0.79	0.16	0.1264	0.91	0.18	0.1638	0.97	0.19	0.1843
	4	0.77	0.15	0.1155	0.78	0.17	0.1326	0.73	0.15	0.1095	0.90	0.19	0.1710	0.94	0.19	0.1786
	6	0.78	0.16	0.1248	0.80	0.18	0.1440	0.74	0.16	0.1184	0.94	0.20	0.1880	0.96	0.19	0.1824
	8	0.81	0.16	0.1296	0.79	0.16	0.1264	0.72	0.15	0.1080	0.91	0.18	0.1638	0.96	0.19	0.1824
	12	0.78	0.16	0.1248	0.76	0.16	0.1216	0.72	0.15	0.1080	0.93	0.19	0.1767	0.99	0.21	0.2079

[z] Means of 12 embryos of each species and hybrids.

Table 5. Means of the embryo length, width, and length x width (mm) across time of mature embryos of rhododendron classes cultured on all 5 media.[z]

Rhododendron classes	Week	Norstog L	Norstog W	Norstog LW	Berlyn L	Berlyn W	Berlyn LW	Raghaven L	Raghaven W	Raghaven LW	Murashige L	Murashige W	Murashige LW	Emsweller L	Emsweller W	Emsweller LW
Evergreen azalea:	1	0.65	0.15	0.0975	0.54	0.15	0.0810	0.66	0.15	0.0990	0.64	0.14	0.0896	0.59	0.14	0.0826
	2	0.66	0.16	0.1056	0.59	0.15	0.0885	0.66	0.15	0.0990	0.64	0.15	0.0960	0.62	0.15	0.0930
	3	0.63	0.17	0.1071	0.58	0.16	0.0928	0.67	0.16	0.1072	0.67	0.16	0.1072	0.63	0.15	0.0945
a. 'Delaware	4	0.62	0.16	0.0992	0.57	0.15	0.0855	0.64	0.15	0.0960	0.64	0.15	0.0960	0.62	0.15	0.0930
Valley	6	0.63	0.17	0.1071	0.60	0.16	0.0960	0.66	0.15	0.0990	0.67	0.16	0.1072	0.61	0.16	0.0976
White' x	8	0.64	0.16	0.1024	0.59	0.15	0.0885	0.66	0.15	0.0990	0.67	0.16	0.1072	0.63	0.15	0.0945
'Glory'	12	0.65	0.17	0.1105	0.59	0.15	0.0885	0.67	0.16	0.1072	0.68	0.16	0.1088	0.63	0.16	0.1008
	1	0.62	0.15	0.0930	0.64	0.16	0.1024	0.58	0.15	0.0870	0.54	0.15	0.0810	0.61	0.13	0.0793
	2	0.62	0.15	0.0930	0.64	0.17	0.1088	0.61	0.15	0.0915	0.54	0.15	0.0810	0.62	0.15	0.0930
b. *R. scabrum*	3	0.62	0.16	0.0992	0.63	0.17	0.1071	0.63	0.16	0.1008	0.55	0.15	0.0825	0.61	0.15	0.0915
	4	0.61	0.16	0.0976	0.63	0.17	0.1071	0.64	0.16	0.1024	0.53	0.15	0.0795	0.60	0.14	0.0840
	6	0.63	0.16	0.1008	0.62	0.18	0.1116	0.64	0.16	0.1024	0.53	0.15	0.0795	0.60	0.15	0.0900
	8	0.63	0.16	0.1008	0.62	0.16	0.0992	0.66	0.16	0.1056	0.53	0.15	0.0795	0.59	0.16	0.0944
	12	0.67	0.17	0.1139	0.65	0.17	0.1105	0.64	0.17	0.1088	0.56	0.15	0.0840	0.60	0.15	0.0900
Scaly rhododendron:	1	0.59	0.15	0.0885	0.70	0.17	0.1190	0.67	0.16	0.1072	0.62	0.15	0.0930	0.65	0.14	0.0910
	2	0.58	0.17	0.0986	0.69	0.16	0.1104	0.65	0.15	0.0975	0.63	0.15	0.0945	0.68	0.14	0.0952
	3	0.62	0.17	0.1054	0.70	0.16	0.1120	0.65	0.16	0.1040	0.63	0.15	0.0945	0.67	0.15	0.1005
R. augustinii	4	0.60	0.16	0.0960	0.70	0.16	0.1120	0.62	0.16	0.0992	0.63	0.15	0.0945	0.65	0.15	0.0975
	6	0.62	0.16	0.0992	0.71	0.17	0.1207	0.66	0.16	0.1056	0.63	0.15	0.0945	0.69	0.15	0.1035
	8	0.62	0.15	0.0930	0.71	0.16	0.1136	0.65	0.15	0.0975	0.64	0.14	0.0896	0.67	0.14	0.0938
	12	0.63	0.16	0.1008	0.71	0.16	0.1136	0.67	0.16	0.1072	0.64	0.15	0.0960	0.71	0.15	0.1065

Table 5. Means of the embryo length, width, and length x width (mm) across time of mature embryos of rhododendron classes cultured on all 5 media.[z] (continued)

Rhododendron classes	Week	Norstog			Berlyn			Raghaven			Murashige			Emsweller		
		L	W	LW	L	W	LW	L	W	LW	L	W	LW	L	W	LW
Non-scaly	1	0.59	0.17	0.1003	0.60	0.16	0.0960	0.63	0.16	0.1008	0.68	0.17	0.1156	0.61	0.16	0.0976
rhododendron:	2	0.60	0.17	0.1020	0.60	0.16	0.0960	0.63	0.17	0.1071	0.69	0.17	0.1173	0.61	0.18	0.1098
	3	0.61	0.18	0.1098	0.62	0.17	0.1054	0.65	0.17	0.1105	0.70	0.18	0.1260	0.63	0.18	0.1134
a. *R. maximum* x 'sefton'	4	0.60	0.17	0.1020	0.61	0.17	0.1037	0.64	0.17	0.1088	0.68	0.17	0.1156	0.62	0.17	0.1054
	6	0.60	0.18	0.1080	0.61	0.17	0.1037	0.63	0.17	0.1071	0.69	0.18	0.1242	0.61	0.18	0.1098
	8	0.61	0.17	0.1037	0.63	0.16	0.1008	0.63	0.16	0.1008	0.69	0.16	0.1104	0.61	0.17	0.1037
	12	0.64	0.18	0.1152	0.63	0.17	0.1071	0.64	0.17	0.1088	0.68	0.17	0.1156	0.62	0.16	0.0992
	1	1.02	0.24	0.2448	1.18	0.25	0.2950	1.15	0.22	0.2530	1.16	0.25	0.2900	1.12	0.22	0.2464
b.	2	1.02	0.24	0.2448	1.19	0.26	0.3094	1.15	0.23	0.2645	1.17	0.24	0.2808	1.15	0.22	0.2530
R. sutchuenense	3	1.02	0.24	0.2448	1.20	0.25	0.3000	1.15	0.23	0.2645	1.17	0.25	0.2925	1.14	0.22	0.2508
	4	1.02	0.25	0.2550	1.18	0.25	0.2950	1.14	0.23	0.2622	1.16	0.26	0.3016	1.14	0.23	0.2622
	6	1.02	0.26	0.2652	1.21	0.27	0.3267	1.18	0.24	0.2832	1.16	0.25	0.2900	1.15	0.23	0.2645
	8	1.04	0.24	0.2496	1.17	0.25	0.2925	1.17	0.23	0.2691	1.15	0.26	0.2990	1.15	0.22	0.2530
	12	1.07	0.26	0.2782	1.20	0.25	0.3000	1.16	0.24	0.2784	1.17	0.26	0.3042	1.18	0.24	0.2832

[z] Means of 12 embryos of each species and hybrids of each rhododendron class.

Table 6. Means of the embryo length, width, and length x width (mm) across time of mature deciduous azalea species and hybrids cultured on all 5 media.[z]

Deciduous azalea	Week	Norstog L	Norstog W	Norstog LW	Berlyn L	Berlyn W	Berlyn LW	Raghaven L	Raghaven W	Raghaven LW	Murashige L	Murashige W	Murashige LW	Emsweller L	Emsweller W	Emsweller LW
	1	0.99	0.28	0.2772	1.19	0.28	0.3332	1.20	0.28	0.3360	1.01	0.27	0.2727	1.08	0.26	0.2808
	2	1.04	0.33	0.3432	1.34	0.32	0.4288	1.58	0.34	0.5372	1.04	0.30	0.3120	1.24	0.30	0.3720
R. calendu-	3	1.04	0.34	0.3536	1.35	0.34	0.4590	1.90	0.36	0.6840	1.06	0.33	0.3498	1.44	0.31	0.4464
laceum	4	1.01	0.39	0.3939	1.33	0.36	0.4788	2.01	0.37	0.7437	1.06	0.33	0.3498	1.66	0.33	0.5478
	6	1.05	0.43	0.4515	1.35	0.40	0.5400	2.33	0.44	1.0252	1.04	0.33	0.3432	1.93	0.34	0.6562
	8	1.04	0.43	0.4472	1.38	0.41	0.5658	2.36	0.46	1.0856	1.04	0.33	0.3432	2.18	0.34	0.7412
	12	1.02	0.43	0.4386	1.40	0.44	0.6160	2.42	0.49	1.1858	1.05	0.33	0.3465	2.18	0.35	0.7630
	1	0.63	0.16	0.1008	0.70	0.16	0.1120	0.78	0.16	0.1248	0.75	0.15	0.1125	0.65	0.15	0.0975
	2	0.67	0.15	0.1005	0.70	0.16	0.1120	0.78	0.15	0.1170	0.75	0.14	0.1050	0.64	0.15	0.0960
	3	0.67	0.16	0.1072	0.69	0.16	0.1104	0.77	0.16	0.1232	0.77	0.15	0.1155	0.65	0.15	0.0975
R. atlanticum	4	0.66	0.17	0.1122	0.69	0.17	0.1173	0.79	0.16	0.1264	0.74	0.15	0.1110	0.66	0.16	0.1056
	6	0.66	0.16	0.1056	0.68	0.17	0.1156	0.77	0.16	0.1232	0.73	0.15	0.1095	0.64	0.16	0.1024
	8	0.67	0.16	0.1072	0.68	0.16	0.1088	0.79	0.16	0.1264	0.74	0.15	0.1110	0.65	0.16	0.1040
	12	0.69	0.18	0.1242	0.70	0.16	0.1120	0.79	0.17	0.1343	0.74	0.15	0.1110	0.64	0.16	0.1024
	1	1.02	0.23	0.2346	0.96	0.21	0.2016	1.13	0.21	0.2373	1.11	0.21	0.2331	1.15	0.22	0.2530
	2	1.09	0.28	0.3052	0.96	0.22	0.2112	1.16	0.24	0.2784	1.11	0.21	0.2331	1.24	0.27	0.3348
R. hybrid	3	1.12	0.28	0.3136	0.94	0.22	0.2068	1.17	0.25	0.2925	1.09	0.21	0.2289	1.24	0.32	0.3968
azalea	4	1.10	0.30	0.3300	0.94	0.22	0.2068	1.16	0.24	0.2784	1.08	0.22	0.2376	1.27	0.35	0.4445
'Night Light'	6	1.11	0.32	0.3552	0.94	0.24	0.2256	1.18	0.25	0.2950	1.10	0.23	0.2530	1.31	0.38	0.4978
	8	1.12	0.31	0.3472	0.96	0.23	0.2208	1.22	0.26	0.3172	1.12	0.23	0.2576	1.39	0.40	0.5560
	12	1.13	0.34	0.3842	0.95	0.24	0.2280	1.23	0.27	0.3321	1.10	0.23	0.2530	1.34	0.43	0.5762

[z] Means of 12 embryos of each species and hybrids.

Table 7. Means of the embryo length, width, and length x width (mm) across time of mature deciduous azalea species and hybrids by cold treatment.[z]

Deciduous azalea	Week	Norstog L	Norstog W	Norstog LW	Berlyn L	Berlyn W	Berlyn LW	Raghaven L	Raghaven W	Raghaven LW	Murashige L	Murashige W	Murashige LW	Emsweller L	Emsweller W	Emsweller LW
Cold	1	1.10	0.24	0.2640	1.13	0.22	0.2486	1.02	0.22	0.2244	1.02	0.20	0.2040	0.98	0.19	0.1862
treatment:	2	1.19	0.30	0.3570	1.27	0.25	0.3175	1.13	0.27	0.3051	1.03	0.20	0.2060	1.23	0.25	0.3075
	3	1.40	0.37	0.5180	1.35	0.27	0.3645	1.47	0.28	0.4116	0.97	0.19	0.1843	1.22	0.30	0.3660
R. calendu-	4	1.61	0.40	0.6440	1.37	0.28	0.3836	1.49	0.29	0.4321	0.97	0.22	0.2134	1.19	0.33	0.3927
laceum	6	1.68	0.45	0.7560	1.37	0.30	0.4110	1.62	0.31	0.5022	0.96	0.21	0.2016	1.50	0.44	0.6600
	8	1.71	0.44	0.7524	1.37	0.32	0.4384	1.69	0.33	0.5577	0.94	0.21	0.1974	1.55	0.45	0.6975
(3 months)	12	1.77	0.46	0.8142	1.43	0.34	0.4862	1.89	0.34	0.6426	0.94	0.21	0.1974	1.60	0.49	0.7840
	1	1.02	0.24	0.2448	1.08	0.22	0.2376	0.96	0.24	0.2304	0.95	0.22	0.2090	0.89	0.22	0.1958
	2	1.31	0.31	0.4061	1.13	0.28	0.3164	0.94	0.34	0.3196	0.95	0.24	0.2280	1.07	0.29	0.3103
R. atlanticum	3	1.55	0.38	0.5890	1.18	0.31	0.3658	0.99	0.32	0.3168	0.92	0.22	0.2024	1.35	0.33	0.4455
	4	1.74	0.40	0.6960	1.24	0.32	0.3968	1.33	0.36	0.4788	0.99	0.28	0.2742	1.62	0.40	0.6480
(3 months)	6	1.88	0.44	0.8272	1.26	0.39	0.4914	1.32	0.39	0.5148	0.99	0.28	0.5772	1.80	0.40	0.7200
	8	1.95	0.46	0.8970	1.26	0.41	0.5166	1.37	0.44	0.6028	1.09	0.29	0.3161	1.92	0.42	0.8064
	12	1.94	0.49	0.9506	1.29	0.42	0.5418	1.37	0.45	0.6165	1.07	0.31	0.3317	1.96	0.46	0.9016
	1	1.39	0.24	0.3336	1.52	0.25	0.3800	1.71	0.25	0.4275	1.72	0.26	0.4472	1.93	0.27	0.5211
	2	1.44	0.28	0.4032	1.71	0.30	0.5130	2.38	0.34	0.8092	2.13	0.34	0.7242	2.83	0.34	0.9622
R. mollis x	3	1.46	0.31	0.4526	1.76	0.31	0.5456	2.79	0.36	1.0044	2.69	0.41	1.1029	2.96	0.36	1.0656
mollis	4	1.45	0.33	0.4785	1.72	0.31	0.5332	2.94	0.36	1.0584	3.07	0.48	1.4736	2.94	0.45	1.3230
	6	1.41	0.32	0.4512	1.71	0.31	0.5301	3.22	0.36	1.1592	3.42	0.55	1.8810	2.98	0.45	1.3410
(25 days)	8	1.44	0.32	0.4608	1.74	0.32	0.5568	[y]	--	--	--	--	--	--	--	--

[z] Means of 12 embryos of each species and hybrids.

[y] Embryos germinated.

Table 8. Percent germination, time of germination (week) and embryo dimensions (mm) at the time of germination of mature deciduous azalea embryos cultured on all 5 media.

Deciduous azalea	Media	Percent Germination	Time of Germination	Embryo dimensions[z] L	W	LW
R. mollis x mollis (matured in refrigerator)	N	0	--	--	--	--
	B	16.7	8	2.96	0.39	1.1544
	R	41.7	4-6	4.70	0.37	1.739
	M	33.3	4-6	5.82	0.47	2.7354
	E	41.7	3-6	3.72	0.58	2.1576
R. calendulaceum	N	0	--	--	--	--
	B	0	--	--	--	--
	R	8.3	4	4.08	0.61	2.4888
	M	0	--	--	--	--
	E	8.3	3	3.22	0.46	1.4812
R. calendulaceum (matured in refrigerator)	N	0	--	--	--	--
	B	0	--	--	--	--
	R	8.3	3	2.99	0.37	1.1063
	M	0	--	--	--	--
	E	0	--	--	--	--

[z] Means of 2 embryos on Berlyn, 4 embryos on Murashige, and 5 embryos on Raghaven or Emsweller for R. mollis x mollis.

Table 9. Means of length and width (mm) of the embryos of all immature species and hybrids of deciduous azalea cultured on all 5 media across time.[z]

Week	Embryo dimensions	Norstog	Berlyn	Raghaven	Murashige	Emsweller
1	L	0.89b[y]	0.95ab	0.86b	0.88b	0.99a
	W	0.207ab	0.221a	0.190bc	0.168d	0.179cd
2	L	0.90a	0.96a	0.95a	0.92a	1.00a
	W	0.194ab	0.210a	0.204a	0.180b	0.188ab
3	L	0.89a	0.94a	0.98a	0.93a	1.00a
	W	0.193ab	0.204a	0.208a	0.177b	0.194ab
4	L	0.87a	0.93a	0.95a	0.92a	1.00a
	W	0.192a	0.200	0.205a	0.179a	0.193a
6	L	0.89a	0.95a	0.99a	0.94a	1.01a
	W	0.193ab	0.209ab	0.218a	0.186b	0.193ab
8	L	0.87a	0.93a	0.96a	0.93a	1.00a
	W	0.192ab	0.201ab	0.219a	0.174b	0.188b
12	L	0.87a	0.93a	0.97a	0.92a	1.00a
	W	0.191ab	0.198ab	0.215a	0.178b	0.191ab

[z] Means of 36 embryos of all three species and hybrids of deciduous azalea.

[y] Means followed by the same letter are not significantly different as determined by Duncan's New Multiple Range Test, 5% level.

Table 10. Means of length and width (mm) of the embryos of all mature species and hybrids of deciduous azalea cultured on all 5 media across time.[z]

Week	Embryo dimensions	Norstog	Berlyn	Raghaven	Murashige	Emsweller
1	L	0.84b[y]	0.96a	1.03a	0.96a	0.96a
	W	0.216a	0.219a	0.215a	0.212a	0.210a
2	L	0.89b	1.02ab	1.17a	0.97b	1.04ab
	W	0.242a	0.237a	0.242a	0.222a	0.241a
3	L	0.90b	1.01b	1.29a	0.98b	1.11ab
	W	0.247a	0.246a	0.255a	0.233a	0.264a
4	L	0.88c	1.00bc	1.33a	0.97bc	1.20ab
	W	0.271a	0.255a	0.258a	0.239a	0.278a
6	L	0.90b	1.01b	1.44a	0.95b	1.30ab
	W	0.287a	0.271a	0.285a	0.244a	0.293a
8	L	0.89b	1.03ab	1.47a	0.96b	1.40ab
	W	0.288a	0.273a	0.296a	0.244a	0.30a
12	L	0.89b	1.03ab	1.48a	0.96b	1.39ab
	W	0.299a	0.288a	0.312a	0.244a	0.314a

[z] Means of 36 embryos of all three species and hybrids of deciduous azalea.

[y] Means followed by the same letter are not significantly different as determined by Duncan's New Multiple Range Test, 5% level.

Table 11. Means of length and width (mm) of the embryos of all the species and hybrids of deciduous azalea matured by the cold of refrigerator and cultured on all 5 media across time.[z]

Week	Embryo dimensions	Norstog	Berlyn	Raghaven	Murashige	Emsweller
1	L	1.20[y]a	1.28a	1.30a	1.30a	1.37a
	W	0.240a	0.234a	0.238a	0.229a	0.232a
2	L	1.34b	1.42b	1.61ab	1.48b	1.88a
	W	0.300ab	0.280ab	0.318a	0.270b	0.301ab
3	L	1.47b	1.48b	1.90ab	1.69ab	2.03a
	W	0.349a	0.299ab	0.324ab	0.295ab	0.341ab
4	L	1.58a	1.48a	2.07a	1.87a	2.10a
	W	0.369ab	0.302b	0.339ab	0.348ab	0.405a
6	L	1.62ab	1.49b	2.22a	2.02ab	2.25a
	W	0.389ab	0.332b	0.351ab	0.377ab	0.430a

[z] Means of 36 embryos of all three species and hybrids of deciduous azalea.

[y] Means followed by the same letter are not significantly different as determined by Duncan's New Multiple Range Test, 5% level.

APPENDIX

Procedure and Chemicals Needed to Prepare the Media

Stock solutions of the chemical contents of each medium were made first to be used for medium mixing. The chemicals were weighed accurately using an electric analytic balance (Mettler H18), dissolved with the help of magnetic stirring or in some cases, with heat. They were added to the solution in the order listed in the tables, with the next one added after the previous element was clearly dissolved.

Table 1a. Medium I (Norstog, 1973)

Stock Solutions:

Stock I	Major elements	Amount mg/l	Weigh out 10X/l mg/l
	$CaCl_2 \cdot 2H_2O$	740	7,400
	KH_2PO_4	910	9,100
	KCl	750	7,500
	$MgSO_4 \cdot 7H_2O$	740	7,400

Stock II	Trace elements	Amount mg/l	100X/500 ml mg/l
	$MnSO_4 \cdot H_2O$	3.0	300
	H_3BO_3	0.5	50
	$ZnSO_4 \cdot 7H_2O$	0.5	50
	$CoCl_2 \cdot 6H_2O$	0.025	2.5
	$CuSO_4 \cdot 5H_2O$	0.025	2.5
	Na_2MoO_4 or replaced by	0.025	2.5
	$(Na_2MoO_4 \cdot 2H_2O)$	0.0294	2.94

Stock III	Fe-Solution	Amount mg/l	100X/500 ml mg/l
	Fe-citrate	10.0	1,000

Stock IV	Vitamins	Amount mg/l	100X/500 ml mg/l
	Inositol (Meso)	50.0	5,000
	Thiamine-HCl	0.25	25
	Ca-pantothenate	0.25	25
	Pyridoxine-HCl	0.25	25

Stock V	Amino Acids	Amount mg/l	20X/400 ml mg/l
	L-glutamine	400	8,000
	L-alanine	50	1,000
	L-cysteine	20	400
	L-arginine	10	200
	L-leucine	10	200
	L-phenylalanine	10	200
	L-tyrosine	10	200

Added ingredients:

Malic Acid	1,000
Sucrose	34,200
Difco Purified Agar	6,000

Norstog Mixing:

a. To 800 ml deionized distilled water add 6 gr Difco purified agar; then autoclave for 20 min at 15 lb pressure and 121^{o}C.

b. Make ammonium malate solution by dissolving 1 gr

malic acid in 30 ml deionized distilled water. Adjust pH to 5.0 with NH_4OH. To this solution (30 ml), add 100 ml major elements (Stock I), 5 ml of trace elements (Stock II), 5 ml Fe-citrate (Stock III), 5 ml vitamins (Stock IV) and 20 ml amino acids (Stock V); adjust pH to 4.9 with NaOH. Add 34.2 gr sucrose and sufficient deionized distilled water to yield 200 ml. Filter through a 0.22 um type GS millipore membrane and add to autoclaved component.

Table 1b. Medium II (Berlyn and Mische, 1965, from Nitsch, 1951)

Stock Solutions:

Stock I	Major elements	Amount mg/l	Weigh out 10X/l mg/l
	$Ca(NO_3)_2 \cdot 4H_2O$	500	5,000
	KNO_3	125	1,250
	$MgSO_4$	125	1,250
	KH_2PO_4	125	1,250
Stock II	**Trace element I**	**Amount mg/l**	**100X/l mg/l**
	Ferric citrate		
	$FeC_6O_5H_7 \cdot 5H_2O$	10	1,000

Stock III	Trace elements II	Amount mg/l	1000X/1 mg/l
	H_2SO_4 (Sp.gr. 1.83)	0.0005cc/1	0.5 cc
	$MnSO_4 \cdot 4H_2O$	3	3,000
	or replaced by		
	$(MnSO_4 \cdot H_2O)$	2.274	2,274
	$ZnSO_4 \cdot 7H_2O$	0.5	500
	H_3BO_3	0.5	500
	$CuSO_4 \cdot 5H_2O$	0.025	25
	$Na_2MoO_4 \cdot 2H_2O$	0.025	25

Added ingredients:

Sucrose	4,000
Difco Purified Agar	6,000

Berlyn Mixing:

a. For 1000 ml, in erlenmeyer liter flask, dissolve 6 gr Difco purified agar in 500 ml (approximately) deionized distilled water with the help of heat over a hot plate and magnetic stirring until the agar completely melts.

b. In a 400 ml beaker, dissolve 40 gr sucrose in 200 ml (approximately) deionized distilled water, add 100 ml of major elements (Stock I), 10 ml trace elements I (Stock II), and 1 ml trace element (Stock III); adjust pH to 5.5 with NaOH. Add this chemical solution and sufficient deionized distilled water to agar solution in the erlenmeyer liter flask to yield 1000 ml.

Table 1c. Medium III (Raghaven and Torrey, 1963)

Stock Solutions:

Stock I	Major elements	Amount mg/l	Weigh out 10X/l mg/l
	KH_2PO_4	60	600
	KCl	42	420
	$MgSO_4 \cdot 7H_2O$	63	630
	$Ca(NO_3)_2 \cdot 4H_2O$	480	4,800
	KNO_3	63	630

Stock II	Trace elements	Amount mg/l	100X/l mg/l
	H_3BO_3	0.56	56
	$MnCl_2 \cdot 4H_2O$	0.36	36
	$ZnCl_2$	0.42	42
	$CuCl_2 \cdot 2H_2O$	0.27	27
	$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	1.55	155

Stock III	Fe-solution	Amount mg/l	100X/l mg/l
	Ferric citrate	4.52	452

Stock IV	Vitamins	Amount mg/l	100X/l mg/l
	Thiamin hydrochloride	0.1	10
	Pyridoxin hydrochloride	0.1	10
	Niacin (nicotinic acid)	0.5	50

Added ingredients:

Sucrose	20,000
Difco Purified Agar	6,000

Raghaven Mixing:

a. Prepare the agar solution as in Berlyn (same amount of agar).

b. In a 400 ml beaker dissolve 20 gr sucrose in 200 ml (approximately) deionized distilled water. Add 100 ml major elements (Stock I), 10 ml trace elements (Stock II), 10 ml Fe-citrate (Stock III), and 10 ml vitamins (Stock IV); adjust pH to 4.9-5.0 with NaOH. Add the final solution and sufficient deionized distilled water to the agar solution in the erlenmeyer liter flask to yield 1000 ml.

Table 1d. Medium IV (Murashige and Skoog, 1962)

Stock Solutions:

Stock I	Major elements	Amount mg/l	Weigh out 10X/l mg/l
	NH_4NO_3	1,950	16,500
	KNO_3	1,900	19,000
	$CaCl_2 \cdot 2H_2O$	440	4,400
	$MgSO_4 \cdot 7H_2O$	370	3,700
	KH_2PO_4	170	1,700
	$MnSO_4 \cdot 4H_2O$	22.3	223
	or replaced by		
	$(MnSO_4 \cdot H_2O)$	16.91	169.1

Stock II	Trace elements	Amount mg/l	1000X/l mg/l
	KI	0.83	415
	H_3BO_3	6.2	3,100
	$ZnSO_4 \cdot 7H_2O$	8.6	4,300
	$CoCl_2 \cdot 6H_2O$	0.025	12.5
	$CuSO_4 \cdot 5H_2O$	0.025	12.5
	$Na_2MoO_4 \cdot 2H_2O$	0.25	125

Stock III	Fe-solution	Amount mg/l	10X/l mg/l
	Na_2EDTA	37.3	373
	$FeSO_4 \cdot 7H_2O$	27.8	278
	Fe-citrate	3.1	31

Stock IV	Vitamins	Amount mg/l	10X/l mg/l
	Pyridoxine-HCl	0.5	5
	Thiamine HCl	0.5	5
	Nicotinamide (Niacin Amide)	0.5	5
	Inositol (Meso)	100	1,000
	Glycine	2.0	20

Added ingredients:

Sucrose	30,000
Difco Purified Agar	6,000

Murashige Mixing:

a. For 1000 ml in a liter erlenmeyer flask, dissolve 6 gr Difco purified agar in 400 ml (approximately)

deionized distilled water with the help of heat over a hot plate and magnetic stirring until the agar completely melts.

b. In a 600 ml beaker dissolve 30 gr sucrose in 200 ml (approximately) deionized distilled water, add 100 ml major elements (Stock I), 1 ml trace element (Stock II), 100 ml Fe-solution (Stock III) and 100 ml vitamins (Stock IV), adjust pH to 5.7-5.8 with NaOH. Add the final solution and sufficient deionized distilled water to the agar solution in the erlenmeyer flask to give 1000 ml.

Table 1e. Medium V (Emsweller, Asen and Uhring, 1962)

Stock Solutions:

Major elements (one stock)	Amount mg/l	Weigh out 10X/1 mg/l
KH_2PO_4	200	2,000
$MgSO_4$	400	4,000
$Ca(NO_3)_2 \cdot 4H_2O$	800	8,000
KNO_3	200	2,000

Added ingredients:

Sucrose	20,000
Difco Purified Agar	6,000

Emsweller Mixing:

a. Prepare the agar solution as in Berlyn (same amount of agar).

b. Prepare the sugar solution as in Raghaven (same amount of sugar), then add 100 ml of the stock of major elements. Add the final solution and sufficient deionized distilled water to the agar solution in the erlenmeyer flask to yield 1000 ml.

GROWTH OF EXCISED RHODODENDRON EMBRYOS IN DIFFERENT TYPES OF MEDIA USED FOR EMBRYO CULTURE

by

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ABSTRACT

Immature and mature embryos of all 4 rhododendron and azalea gardening class types were excised and cultured in the dark at 25°C on 5 types of media used for embryo culture (Norstog, Berlyn, Raghaven, Murashige, and Emsweller). Embryo size was measured at 1, 2, 3, 4, 6, 8 and 12 weeks to see the change across time. Small embryos of 0.5-1 mm in length from all rhododendron and azalea classes did not grow or germinate on the 5 media. Embryos of mature deciduous azalea species and hybrids larger than 1 mm in length, particularly R. calendulaceum, showed the most embryo growth and differentiation leading to some germination on Raghaven and Emsweller media. Embryos matured by cold treated immature seeds at 3-4°C until pods became brown and split-open, grew much better than mature embryos in culture after excision. Mature embryos of 3 month cold treated immature R. calendulaceum seeds grew best on Raghaven and Emsweller even though just a low percent germination occurred on Raghaven. Norstog and Emsweller appeared to support embryo growth of 3 month cold treated immature R. atlanticum seeds without any germination. Mature embryos of 25 days cold treated R. mollis x mollis seeds grew and developed successfully on Raghaven, Emsweller, and Murashige with high percent germination; they had slight growth on Norstog and Berlyn but a few embryos germinated late on Berlyn. The mature embryos of species and hybrids of deciduous azalea differ in their nutritional requirements due to their

own genetic characteristics, but generally they seemed to respond best on simple media as Emsweller. Rhododendron tissue seemed to be sensitive to the concentrations of salts, compounds in the medium especially Murashige, which caused browning to small rhododendron seedlings after one month exposure to light intensity of 700-1,000 ft.c.